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Study of Salt Stress Tolerance in Spelt

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Study of Salt Stress Tolerance in Spelt

(*Triticum aestivum var. Spelta*)

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Estudo da Tolerância da Espelta sob Stress Salino
(*Triticum aestivum var. Spelta*)

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Abstract

World population is increasing at an alarming rate while food productivity is decreasing due to the effect of various abiotic stresses. Soil salinity is one of the most important abiotic stress and a limiting factor for worldwide plant production. In addition to its important effects on yield, salt stress affects numerous cellular activities, including cell wall composition, photosynthesis, protein synthesis, ions and organic solutes. Up to 20% of the irrigated arable land in arid and semiarid regions is already salt affected and is still expanding. Improving salt tolerant varieties is of major importance, and efforts should be focused on finding adaptive mechanisms which are involved in salinity tolerance. In this study, several spelt wheat (*Triticum aestivum* var. Spelta) genotypes and one cultivar of modern bread wheat were used to screen them for salt tolerance. Spelt is an old-European cereal crop currently attracting renewed interest as a food grain because it is said to be harder than wheat and requires less fertilizer. Spelt wheat is also becoming very attractive genetic source by plant breeders due to its wide adaptation ability to various stressful conditions such as soil salinity. In this study morphological parameters (e.g., leaf appearance; shoot elongation), dry matter production, mineral nutrients (especially Na and K), and activity of antioxidative enzymes were measured to select superior genotypes of spelt for salt tolerance. The results showed that Spelt genotype Sp41 is a salt sensitive genotype and genotypes Sp69, Sp96 and Sp912 are good candidates for salt tolerant genotypes.

Keywords: Stress, Soil Salinity, Salt Tolerance, Spelt (*Triticum aestivum* var. Spelta).

Resumo

A população mundial está a crescer a um ritmo elevado, e por outro lado, a produtividade de alimentos está a baixar devido ao efeito de vários stresses abióticos. A salinidade dos solos é um desses stresses que causa mais limitações para a produção agrícola em todo o mundo. Além dos seus efeitos a nível de rendimento, o stress salino afecta inúmeras actividades celulares, incluindo a composição da parede celular, a fotossíntese, a síntese de proteínas, iões e solutos orgânicos. Até 20% da terra arável irrigada em regiões áridas e semi-áridas já foi afetada pelo sal (e esta percentagem continua a crescer). Melhorar espécies para que estas sejam tolerantes ao sal é de grande importância, e investigação deve ser feita na procura de mecanismos adaptativos envolvidos na tolerância à salinidade. Neste trabalho, vários genótipos de espelta (*Triticum aestivum* var. Spelta) e um cultivar de trigo moderno foram estudados para encontrar genótipos com tolerância ao sal. A Espelta é um cereal cultivado na Europa antiga que está actualmente a voltar a atrair interesse como alimento para cultivo, pois a espelta é dita ser mais resistentes (a factores externos) do que o trigo e exige menos fertilizantes. Está também a atrair atenções a nível genético para melhoramentos de outros cultivares, devido à sua grande capacidade de adaptação a várias condições de stresse, como a salinidade dos solos. Neste estudo, parâmetros morfológicos (por exemplo, aparecimento de folhas; comprimento das folhas), produção de matéria seca, nutrientes minerais (principalmente sódio e potássio), e a actividade de enzimas antioxidantes foram medidos para seleccionar genótipos de Espelta com maior aptidão para a tolerância à salinidade. Os resultados mostraram que o genótipo Sp41 é um genótipo sensível à salinidade e os genótipos Sp69, Sp96 e Sp912 são bons candidatos para a tolerância à salinidade.

Palavras-chave: Stress, Salinidade dos solos, Tolerância ao sal, Espelta (*Triticum aestivum* var. Spelta).

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Abbreviations

AA	Ascorbic Acid
APX	Ascorbate Peroxidase
CAT	Catalase
EC	Dehydroascorbate Reductase
FENS	Faculty of Engineering and Natural Sciences
FW	Fresh Weight
GR	Glutathione Reductase
GSSG	Oxidized Glutathione
ICP	Inductively coupled plasma
ICP-OES	Inductively coupled plasma optical emission spectrometry
$NADP^+$	Nicotinamide Adenine Dinucleotide Phosphate
NADPH	Nicotinamide Adenine Dinucleotide Phosphate Oxidase
NBT	p-nitro blue tetrazolium chloride
POD	Peroxidase
ROS	Reactive Oxygen Species
SOD	Superoxide Dismutase

1. Introduction

1.1. Background

In Earth's ecosystem, humans are consumers and consume primarily plants. In the future, human population will continue to rise (Mahajan and Tuteja, 2005) and also their standard of living had increased; nowadays they consume more animals and animal products. Such diets consume more agricultural resources. During the past 10,000 years agricultural production has become gradually more intense (Chrispels and Sadava, 2003). Also, the growth and productivity of plants are greatly affected by various environmental stresses (Moud and Maghsoudi, 2008). The effect of these environmental stresses on crop plants is a topic that is receiving increasing attention because of the potential impacts of climate change on rainfall patterns, temperature extremes and salinization of agricultural lands by irrigation and the overall need to maintain or increase agricultural productivity on marginal lands (Oliveira, *et al*., 2013). Crop response to stress situations further depends on the intensity and duration of stress, plant genotype, developmental stage and environmental factors that cause stress (Aliyev, 2012). Among these, soil salinity is one of the most important abiotic stress and limiting factor for worldwide plant production (Moud and Maghsoudi, 2008; Xiong *et al.,* 2002). Nearly 20% of the world's cultivated area and nearly half of the world's irrigated lands are affected by salinity. Processes such as seed germination, seedling growth and vigour, vegetative growth, flowering and fruit set are adversely affected by high salt concentration, ultimately causing diminished economic yield as well as the quality of the related products (Sairam and Tyagi, 2004).

Improving salt tolerant varieties on the other hand, is of major importance, and efforts should be focused on finding adaptive mechanisms which are involved in salinity tolerance. This may lead to find gene sources as well as morphological and physiological parameters for screening large number of genotypes for salt tolerance. Hence, a detailed understanding of the basic mechanisms involved in the plant salt tolerance is an important prerequisite to improve the performance of crop plant in saline soils.

In this context the aim of this study was to select superior genotypes of spelt for salt tolerance and to understand plant factors contributing to salt tolerance.

1.2. The Importance and Origins of Agriculture

Domestication of plants and animals is the major factor underlying human civilization and is a gigantic evolutionary experiment of adaptation and speciation, generating incipient species (Peng *et al*, 2011). All people on earth today are sustained by agriculture and no other species is a farmer. Essentially all of the arable land in the world is under cultivation. Yet agriculture began just a few thousand years ago, long after the appearance of anatomically modern humans (Chrispels and Sadava, 2003). The beginning of agriculture around 10 000 years ago has repeatedly been seen as the major transition in the human past, a changeover from the natural environment in control of humans, to humans in control of the natural environment. Before agriculture, humans were hunter-gatherers, dependent on wild resources for their nutritional requirements, which led to a largely nomadic lifestyle dictated by the annual cycle of animal and plant availability. The cultivation of plants and the husbandry of animals enabled humans to exert a measure of control over their food resources, protecting them from climatic and environmental uncertainty (Brown *et al*., 2008).

The modern human diet is very different from that of closely related primates and, almost certainly, early hominids (Gordon 1987). Though there is controversy over what humans ate before the development of agriculture, the diet certainly did not include cereals in appreciable quantities. The storage pits and processing tools necessary for significant consumption of cereals did not appear until the Neolithic (Chrispels and Sadava, 2003).

Agriculture began independently in several parts of the world at about the same time (Brown *et al*., 2008). Ample phytogeographical, molecular, archeobotanical, and genetic evidence points to a small 'core area' in southeastern Turkey and northern Syria as the cradle of agriculture (Peleg *et al*., 2011).

It is known that the fourth major centre of domestication was the 'Fertile Crescent,' a region of southwest Asia comprising the valleys of the Tigris, Euphrates and Jordan rivers and their adjacent hilly flanks (Fig. 1.1) (Sayre, 2013; Brown et al., 2008)

Fig. 1.1- 'Fertile Crescent,' in green (a region of southwest Asia comprising the valleys of the Tigris, Euphrates and Jordan rivers.). From Sayre, 2013.

1.2.1. Wheat

Wheat is a major cereal crop in many parts of the world and it is commonly known as king of cereals. It belongs to *Poaceae* family and globally wheat is the second most produced food among the cereal crops (Datta *et al*., 2009). It is one of the most important grain crops in the world and consists mainly of two types: common wheat and durum wheat. Common or bread wheat (*Triticum aestivum*) accounts for some 95% of all the consumed wheat in the world today; the other five percent is durum or hard wheat (*Triticum durum*), used in macaroni and low-rising bread (Peng *et al*, 2011).

Wheat is one of the Neolithic founder crops, domesticated alongside other cereals. Today, wheat is the world's most important food crop, providing about one-fifth of the calories consumed by man, with approximately 620 million tons in 2006 and 681 million tons in 2011 produced worldwide (Dubcovsky and Dvorak, 2007; Brenchley *et al.*, 2012; Peleg *et al*., 2011). The consumption of wheat increases each year, it is estimated that global wheat production between 2010 and 2020 will rise by 40% (Aliyev, 2012).

Bread and durum wheat are both domesticated forms of wild emmer wheat. Bread wheat (*T. aestivum*) originated from a cross between domesticated emmer wheat (*T. dicoccum*) and the goat grass (*Aegilops tauschii*). Wild emmer wheat has the same genome formula as durum wheat and has contributed two genomes to bread wheat, and is central to wheat domestication (Fig. 1.2) [1] (Peng *et al*, 2011).

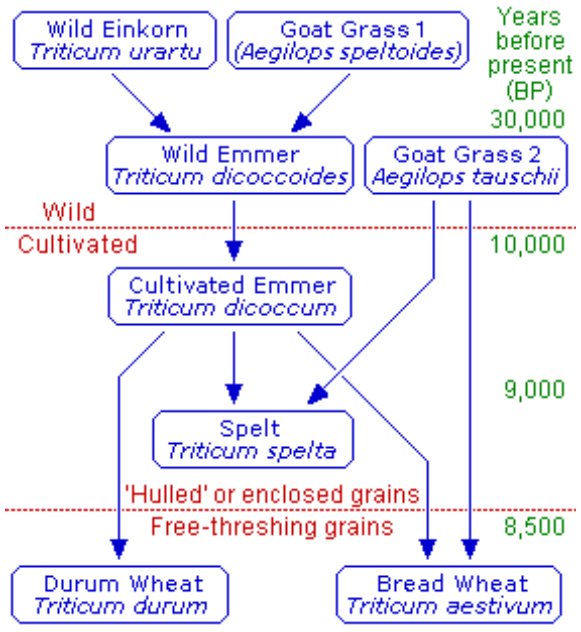

Fig. 1.2 - The evolution of wheat. [1]

1.2.2. Spelt

Spelt (*Triticum aestivum var. Spelta*) is an old-European cereal crop preceded only by Emmer (*T. dicoccum*) and Einkorn (*T. monococcum*) (Campbell, 1997; Neeson, 2011).

For many years, it was believed that bread wheat had evolved from spelt by mutations that changed the form of the ear. Newer scientific research now suggests that it evolved independently about 8 500 years ago but from the same two ancestors, Cultivated Emmer and a Goat Grass (Fig. 1.2) (Marcussen *et al.*, 2014). This created a free-threshing hybrid that differed from Spelt by the ear being roughly square in section, with more grains and a tougher rachis [1].

In Fig .1.3 it is possible to see the differences between spelt wheat and bread wheat (Campbell, 1997).

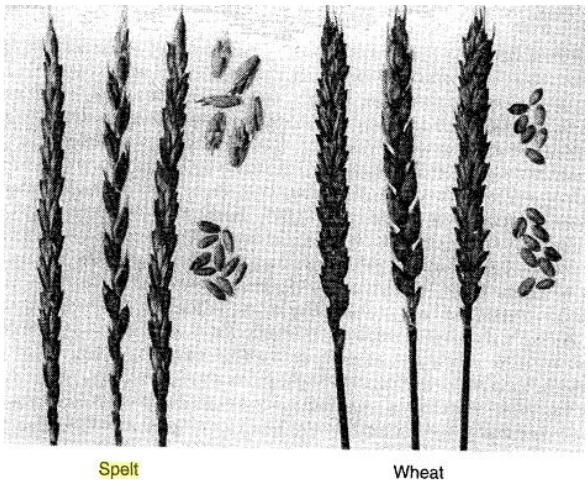

Fig .1.3- Differences between spelt and wheat. From Campbell, 1997.

Spelt is currently attracting renewed interest as a food grain in Europe and also in North America. Although spelt was one of the major feed and food grains of ancient Europe, it is now considered a minor crop (Campbell, 1997). In the 20th century, spelt was virtually replaced by wheat, which produces higher yields and is easier to thresh. However, since spelt is said to be harder than wheat and requires less fertilizer organic farmers made it more popular again towards the end of the century. It is now mainly grown in Central Europe and Northern America and has found a new market as a health food, because of its richness in diverse of nutritional compounds (Neeson *et al.*, 2011). The most common use for spelt is as a substitute for wheat flour in breads, pasta, cookies, crackers, breakfast cereal, cakes, muffins, mixes for breads, pancakes and waffles, and in animal feedstuffs. Spelt has high protein content and makes high-quality bread. It can also be used for making beer and for spelt rice. (Neeson,2011). Spelt wheat is also becoming very attractive genetic source by plant breeders

due to its wide adaptation ability to various stressful conditions such as soil salinity (Neeson *et al*., 2011). The main European spelt producing countries are Italy, France, Germany and Spain.

1.3. Nowadays Agriculture

During the past 10,000 years agricultural production has become gradually more intense (Chrispels and Sadava, 2003). As the world population continues to grow, the availability of renewable freshwater resources for agriculture will decrease, and simultaneously the area of irrigated land will increase in the attempt to satisfy the need for more food (Jaarsma *et al*., 2013). Irrigation systems are particularly prone to salinization (Munns, 2002), once that when the plants use the water, the salts are left behind in the soil and eventually begin to accumulate soils (Oliveira *et al*., 2013). About half the existing irrigation systems of the world are under the influence of salinization, alkalization or waterlogging (Munns, 2002). The area of salt affected soils will rapidly expand in the near future (Jaarsma *et al*., 2013).

Fig. 1.4 The effect of soil salinity on the growth of wheat.
From Chrispels and Sadava, 2003

Soil salinity is a major constraint to food production because it limits crop yield and restricts use of land previously uncultivated (Fig. 1.4) (Yokoi *et al*., 2002).The saline accumulation in the soil it is due to some processes such as: the combined effect of meager rainfall, high evaporation, the presence of salt-bearing sediments, and in many places, particularly river valleys and other low-lying areas, the occurrence of shallow, brackish groundwater which gives rise to saline soils (Oliveira, *et al*., 2013) and as already mentioned irrigation systems. However, salinization can be managed by changed farm management practices. In irrigated agriculture, better irrigation practices, such as drip irrigation, to optimize use of water can be

employed. In rain-fed agriculture, practices such as rotation of annual crops with deep-rooted perennial species may restore the balance between rainfall and water use, thus preventing rising water tables bringing salts to the surface. All such practices will rely on a high degree of salt tolerance, not only of the perennial species used to lower a saline water table, but also of the crops to follow, as some salt will remain in the soil (Munns, 2002).

Increased salt tolerance of crops is needed to sustain food production in many regions in the world. In irrigated agriculture, improved salt tolerance of crops can lessen the leaching requirement, and so lessen the costs of an irrigation scheme (Munns, 2006).

1.4. Plant Nutrition in Adverse Soil Conditions

Plant nutrition refers to the need for basic chemical elements for plant growth. Plant growth requires not only carbon dioxide and oxygen from the air but also water and mineral nutrients from the soil. Soil has been called the "placenta of life," because it supplies essential nutrients to all land plants, and the plants in turn feed all the terrestrial ecosystems (Chrispels and Sadava, 2003).

Poor management of natural resources (deforestation and misuse of agricultural land) has led to extensive soil degradation all over the world. Soil degradation is defined as a decline in soil quality that impairs the soil`s current or potential capacity to produce crops. It includes physical, chemical, and biological deterioration. Soils have been and are being degraded by erosion, salinization, compaction, nutrient losses, pollution, and biological deterioration. Three quarters of the area degraded by inappropriate agricultural practices, overgrazing, and deforestation are in the developing world (Chrispels and Sadava, 2003). Near 40% of the agricultural land has been affected by soil degradation (Cakmak, 2002).

1.4.1. Soil Salinity

Soil salinity is a prevalent abiotic stress that limits the productivity and geographical distribution of plants (Radi *et al*., 2013). The term “salinity” refers to the presence in soil and water of electrolytic mineral solutes in concentrations that are harmful to many agricultural crops (Oliveira *et al*., 2013), which means that high concentrations of soluble salt in the soils is a major constraint to crop productivity, especially in the arid and semi-arid areas of the world (Alhagdow *et al*., 1999). Excessive salts in soil affect all major living processes such as growth, photosynthesis, protein, and lipid metabolism (Radi *et al*., 2013). Accordingly, plants needs to regulate water transport under salinity stress because a sufficient amount of water is

indispensable for cells to maintain growth and vital cellular functions (namely, photosynthesis and metabolisms) (Horie *et al*., 2012).

The Fig. 1.5 is showing that deleterious effects of salinity on plant growth are associated with low osmotic potential of soil solution, nutritional imbalance, specific ion effect, or a combination of these factors (Evelin *et al.,* 2009).

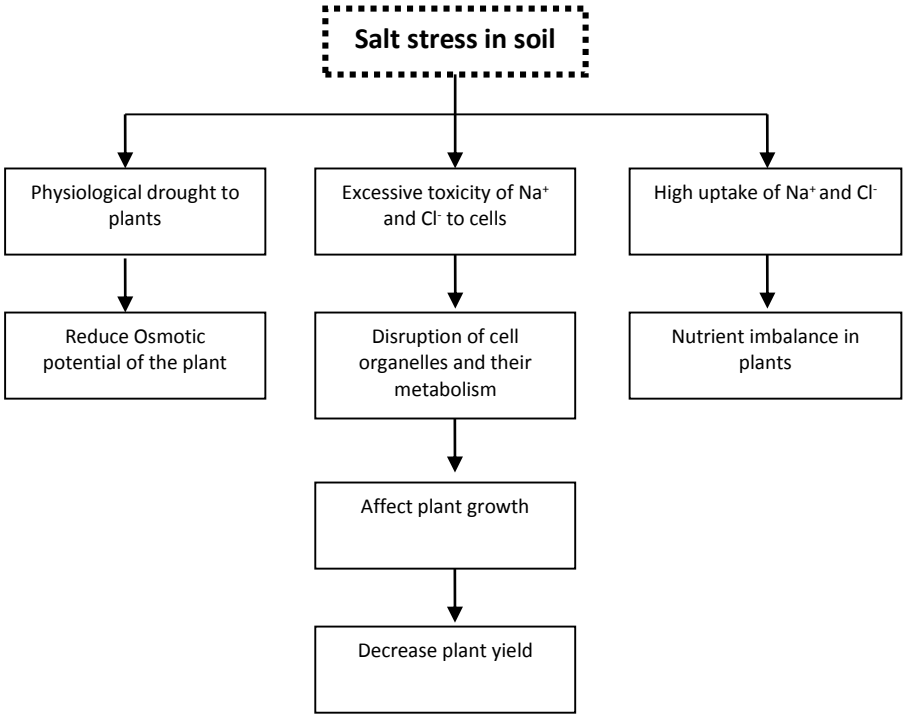

Fig. 1.5 - Effects of saline soil on plants. Adapted from Evelin *et al*., 2009.

1.5. Saline Stress and Plant Response

As already mentioned salt stress is one of the common abiotic stress threats to agriculture and can significantly reduce crop productivity (Wang *et al*., 2013), once that salt stress reduces water potential and causes ion imbalance or disturbances in ion homeostasis and toxicity. This altered water status leads to initial growth reduction (Fig. 1.6) and limitation of plant productivity (Parida *et al*., 2004). Also, since high salinity causes both hyperionic and hyperosmotic stress (Radi *et al*., 2013) growth suppression is directly related to total concentration of soluble salts or osmotic potential of soil water (Parida *et al*., 2004) and can lead to plant demise (Radi *et al*., 2013). In general salt stress affects numerous cellular activities, including cell wall composition, photosynthesis, protein synthesis, ions and organic solutes content (Russo *et al*., 2000; Moud and Maghsoudi, 2008) which results in a reduction in

biomass production, a decrease in shoot length (Fig. 1.7), induction of senescence response or earlier plant death (Moud and Maghsoudi, 2008; Jaarsma *et al*., 2013).

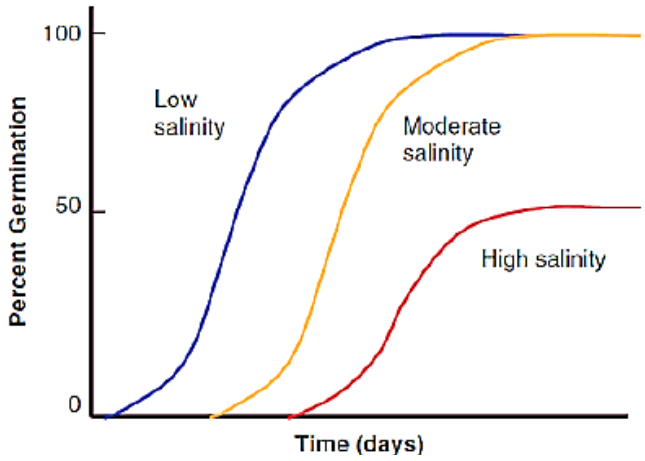

Fig. 1.6 - Salinity effects on relationship between percent germination and time after water addition at low, moderate and high salinity. From Oliveira, *et al*., 2013.

Under salt stress, plants have to cope with stress imposed by the low external water potential and with ion toxicity due to accumulation of ions inside the plants (Tammam *et al*., 2008). It is necessary to know whether plant´s growth is being limited by the osmotic effect of the salt in the soil, or the toxic effect of the salt within the plant. In the simplest analysis of the response of a plant to salinity stress, the reduction in shoot growth occurs in two phases: a rapid response to the increase in external osmotic pressure, and a slower response due to the accumulation of Na^+ in leaves (ionic stress) (Table 1.1) (Munns, 2008).

Table 1.1 - The effects of salinity stress on plants.

Effect of stress	Osmotic stress	Ionic stress
Speed of onset	Rapid	Slow
Primary site of visible effect	Decreased new shoot Growth	Increased senescence of older leaves

The former stress immediately comes over plants in accordance with a rise in salt levels outside the roots, which leads to inhibitions of water uptake, cell expansion and lateral bud development. The latter stress phase develops later when toxic ions such as Na^+ accumulate in excess in plants particularly in leaves over the threshold, which leads to an increase in leaf mortality with chlorosis and necrosis, and a decrease in the activity of essential cellular metabolisms (Horie *et al*., 2012).

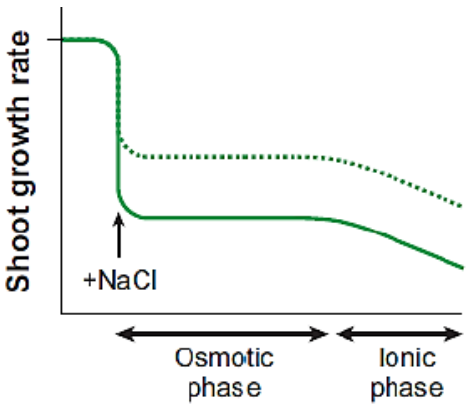

Fig. 1.7 - Growth responses to salt stress from Oliveira, *et al*., 2013.

The plant response to salinity consists of numerous processes that must function in coordination to alleviate both cellular hyperosmolarity and ion disequilibrium (Yokoi *et al*., 2002).

The need to develop crops with high salinity tolerance has increased considerably within the last few decades (Ashraf and O'leary, 1996).

1.5.1. Salt Tolerance

Salt tolerance is the ability of plants to grow and complete their life cycle on a substrate that contains high concentrations of soluble salt (Parida *et al*., 2004). Plants that can survive on high salt medium and grow well are called halophytes but most of the plants are glycophytes and cannot tolerate salt-stress (Sairam and Tyagi, 2004). Many plants develop biochemical and molecular mechanisms either to exclude salt from their cells or to tolerate its presence within the cells. Biochemical strategies include (i) selective accumulation or exclusion of ions, (ii) control of ion uptake by roots and transport into leaves, (iii) compartmentalization of ions at the cellular and whole-plant levels, (iv) synthesis of compatible solutes, (v) change in photosynthetic pathway, (vi) alteration in membrane structure, (vii) induction of antioxidative enzymes, and (viii) induction of plant hormones (Parida *et al*., 2004). In Fig. 1.8 it is represented a schematic summary of the stresses that plants suffer under high salinity growth condition and the corresponding responses that plants use in order to survive these detrimental effects.

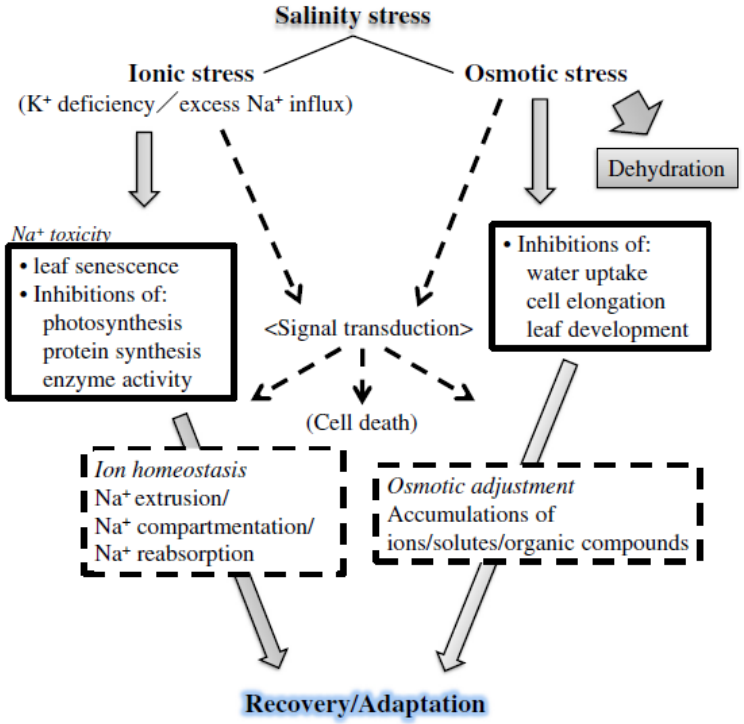

Fig. 1.8 – A schematic summary of the stresses that plants suffer under high salinity growth condition and the corresponding responses that plants use in order to survive these detrimental effects. From Horie *et al*., 2012.

1.5.1.1. Salt Movement Through Plants

Dissolved in solution, salt reduces the availability of water to the plant (Lockhart, 2013). Movement of salt into roots and to shoots is a product of the transpirational flux required to maintain the water status of the plant. Unregulated, transpiration can result in toxic levels of ion accumulation in the aerial parts of the plant (Hasewaga, 2000).

In the long distance water transport from roots to shoots, evaporation is one of the main motive forces for the water movement. Salinity/osmotic stress directly or indirectly via hormonal regulation induces a stomatal closure, which leads to a reduction in the evaporation and overall water transport (Horie *et al*., 2012).

1.5.1.2. Mechanism of Salt Tolerance

The mechanisms of salinity tolerance fall into three categories:

1. **Tolerance to osmotic stress.** The osmotic stress immediately reduces cell expansion in root tips and young leaves, and causes stomatal closure (Munns, 2008). Under osmotic stress, an important consideration is to accumulate osmotically active compounds called osmolytes in order to lower the osmotic potential. These are referred to as compatible metabolites because they do not apparently interfere with the normal cellular metabolism (Sairam and Tyagi, 2004).

2. **Na^+ exclusion from leaf blades**. Na^+ exclusion by roots ensures that Na^+ does not accumulate to toxic concentrations within leaves. A failure in Na^+ exclusion manifests its toxic effect after days or weeks, depending on the species, and causes premature death of older leaves (Munns, 2008). Under salt stress, tolerant species may limit Na^+ uptake but maintain high foliar K^+ levels. High foliar K/Na ratio was suggested as an indication of salinity tolerance (Alhagdow *et al*., 1999).

3. **Tissue tolerance**: In order to avoid Na^+ toxicity, the plant cell may either transport the ions outside the cell or store them inside the vacuole, processes mediated by specialized proteins. Some of them belong to the family of Na^+/H^+ exchangers, which can be located in the plasma membrane or vacuole. Na^+/H^+ antiporters have been identified in several mammals, bacteria and plants. These transporters play roles in pumping out Na^+ from the cytoplasm by exchanging it for H^+ at the expense of the proton gradient generated by specialized pumps in the cell and vacuolar membrane.

This mechanism allows plant cells to accumulate Na^+ in the vacuole and therefore maintain the appropriate ion concentration in the cytoplasm (Baltierra, 2013) thereby protecting the cytoplasm from ion toxicity and avoiding buildup in the cell wall which would cause dehydration. Ion compartmentalization in the vacuole requires energy-dependent transport which is the cost to the plant of coping with stress (Leksungnoen, 2012).

In the following table (Table 1.2) you can see some mechanisms of salinity tolerance, organized by plant processes and their relevance to the three components of salinity tolerance (Munns, 2008):

Table 1.2 - Mechanisms of salinity tolerance.

	Osmotic Stress	**Ionic Stress**	
Process Involved	**Osmotic Tolerance**	**Na^+ Exclusion**	**Tissue Tolerance**
Sensing and Signaling in Roots	Modification of long-distance signaling	Control of net ion transport to shoot	Control of vacuolar loading
Shoot Growth	Decreased inhibition of cell expansion and lateral bud development	-	Delay in premature senescence of old (carbon source) leaves
Photosynthesis	Decreased stomatal closure	Avoidance of ion toxicity in chloroplasts	Delay in ion toxicity in chloroplasts
Accumulation of Na^+ in Shoots	Increased osmotic adjustment	Reduced long distance transport of Na^+	Reduced energy spent on Na^+ exclusion
Accumulation of Na^+ in Vacuoles	Increased osmotic adjustment	Increased sequestration of Na+ into root vacuoles	Increased sequestration of Na^+ into leaf vacuoles
Accumulation of Organic Solutes	Increased osmotic adjustment	Alteration of transport processes to reduce Na^+ accumulation	Accumulation of high concentrations of compatible solutes in cytoplasm

In addition, soil salinity stress causes in-planta accumulation of reactive oxygen species (ROS), which can result in oxidative stress and cellular damage. (Lockhart, 2013) ROS such as superoxide radical ($O2^{\bullet -}$), hydrogen peroxide (H_2O_2) and hydroxyl radical ($OH^{\bullet}$) are responsible for the damage to membranes and other essential macro-molecules such as photosynthetic pigments, protein, DNA and lipids (Sairam and Srivastava, 2002). For mitigating their

deleterious effects, plants have developed antioxidant enzymes such as superoxide dismutase (SOD), ascorbate peroxidase (APX), glutathione reductase (GR), catalase (CAT), peroxidase (POD) and non-enzymatic scavengers like glutathione, ascorbic acid and carotenoids (Mandhania *et al*., 2006).

The primary scavenger is SOD, which converts $O2^{\bullet -}$ to H_2O_2. This toxic product of SOD reaction is eliminated by APX in association with dehydroascorbate reductase (EC) and GR, the later two help in regeneration of ascorbic acid (AA). H_2O_2 is also scavenged by CAT, though the enzyme is less efficient than APX/GR system (Sairam and Srivastava, 2002). Glutathione reductase is important to scavenge and remove these toxic products before cellular damage occurs because it plays an essential role in the protection of chloroplasts against oxidative damage by oxidation of essential thiol groups, inactivating these enzymes (Gamble and Burke, 1984).

2. Materials and Methods

All experiments were conducted in greenhouse of Faculty of Engineering and Natural Sciences of Sabancı University, Tuzla/Istanbul, Turkey. The soil used was collected from Central Anatolia region of Turkey. This soil is characterized as highly calcareous and semi-arid, because this area is the driest region in Turkey, with an annual precipitation of 325 mm (Cakmak *et al.*, 1996). Is also known to have deficiency in lot of nutrients (Bagci *et al.,* 2007; Cakmak, 2008). All experiments had completely randomized and full factorial designs.

2.1. Plant Culture and Treatments

Three experiments were conducted as described below:

2.1.1. 1st Experiment

Eight spelt wheat genotypes (Sp2, Sp41, Sp492, Sp563, Sp732, Sp757, Sp804 and Sp912) and one cultivar of modern bread wheat (cv. Adana99) from the germoplasm bank of Sabancı University, Tuzla/Istanbul, Turkey, were used in screening studies for salt tolerance. The reason why cv. Adana99 will be used as reference genotype was because is one of the most used seeds in Anatolian soil, due to have better growth rates and high yields in this type field (Mazid *et al.*, 2009). Two different treatments (salt tolerance and the respective control) were applied and each genotype was cultivated in 3 independent pots by using about 10 randomized seeds per pot. Each pot was filled with about 2.2Kg of soil and the soils were fertilized with 200 ppm of N, 100 ppm of P, 5 ppm of Zn and 5 ppm of Fe by using $Ca(NO_3)_2$, KH_2PO_4, $ZnSO_4$, and FeEDTA, respectively. To study salt tolerance, 2500 ppm of NaCl was added to the pots used for salt stress treatment. Then, plants were grown with limited and sufficient water supply for 30 days, thereafter harvested (e.g., after 50 days of growth under greenhouse conditions).

2.1.2. 2nd Experiment

Eight spelt wheat genotypes (Sp53, Sp67, Sp69, Sp92, Sp96, Sp225, Sp382 and Sp801) and one cultivar of modern wheat (Adana99), used as reference genotype, from the germoplasm bank of Sabancı University, Tuzla/Istanbul, Turkey, were selected to screen them for salt tolerance. The experimental procedure used in the first experiment was also used in this experiment with the following differences: Before sowing, the seeds of all genotypes were vernalized for 3 weeks at 3-4^{0}C in order to achieve better germination and seedling growth. Since the 2500 ppm NaCl treatment used in the first test caused severe salt stress, in this second test 2000

ppm NaCl was used. Vernalization is the process by which prolonged exposure to cold temperatures promotes flowering (Amasino, 2004). Optimum vernalization temperatures, with some exceptions, range from 3-8 °C and the time required for complete vernalization depends of the specie. The length of vernalization treatment required for complete vernalization is related to whether a species has an obligate or facultative vernalization requirement. Many crops of the biennial plants stay vegetative without cold exposure; the cold requirement is therefore called obligatory. On the other hand, for some species, vernalization has only a furthering effect on flower induction. The cold requirement for those is called facultative (Kaymak and Güvenç, 2010).

2.1.3. 3rd Experiment

The genotypes showing best and lowest performance under salt stress treatments of the 1st and 2nd experiments were selected and used in this 3rd experiment. These genotypes were as following: Sp41, Sp67, Sp69, Sp92, Sp96, Sp563, Sp732 and Sp912. The treatments used in this 3rd test were the same of the treatments applied in the 2nd test.

2.2. Determination of Dry Matter Production

At harvest, only shoot samples were collected, washed in deionized water, placed in paper bags and storage inside the oven at 50°C during 3 days. Then, each sample was weighed (Sartorius CP3202S, d=0.01g) to determination of dry matter.

2.3. Determination of Mineral Nutrients

Whole shoot and root samples were dried at 70 °C. Dried samples were milled to fine powders in an agate vibrating cup mill (Pulverisette 9; Fritsch GmbH; Germany) during around 1 minute at 750rpm, digested and sent to ICP analysis for determination of macro (K, Ca, Na, P, S and Mg) and micronutrients (Zn, Fe, Mn, Cu, B and Al).

To digest, each sample were weighed (0.30g ± 0.10g) and transferred to a digestive tube, which was filled with 2mL of 30% H_2O_2 and 5mL of 65% HNO_3, and then all samples were acid digested in a closed-vessel microwave system, (MarsExpress; CEM Corp., Matthews, NC, USA) After this process, 13mL of double-deionized water were added in each tube (total volume =20mL) and then all samples were filtered and storage. A blank was added to our set of samples, and also a reference of Tomato Leaf (NIST 1573a) (0.20g ± 0.00g). Inductively coupled plasma optical emission spectrometry (ICP-OES; Vista-Pro Axial; Varian Pty Ltd, Mulgrave, Australia) was used to determine the mineral concentrations of the samples. Measurements

were checked by using certified standard reference materials obtained from the National Institute of Standards and Technology (Gaithersburg, MD, USA).

2.4. Determination of Enzymatic Activity

A sample of approximately 0.5g (Sartorius CP3202S, d=0.01g) of fresh leaves were collected from each pot and kept at -80 °C. All samples were milled with help of liquid nitrogen and quartz powder in a porcelain mortar. Then, 5 mL of 50 mM potassium phosphate (K-P) buffer solution was added to the samples. The K-P buffer was prepared by mixing 50 mM KH_2PO_4 and 50 mM K_2HPO_4 and the pH was adjusted to 7.6. Then, 0.1 mM EDTATitriplex-III was added to this mixture for the homogenization step. The homogenates were then centrifuged at 15000g for 30 min, and the supernatants were used for protein and enzyme analysis. Protein concentrations in the crude extracts were measured by using the Bradford assay as described by Bradford (1976). Superoxide dismutase activity was measured by a slightly modified version of the photochemical method described by Giannopolitis and Ries (1977). This assay is based on the inhibition of the photochemical reduction of p-nitro blue tetrazolium chloride (NBT) by SOD and its spectroscopic measurement at 560 nm. One tube of reaction mixture contains 500 µL 50 mM Na_2CO_3, 500 µL 12 mM Lmethionine, 500 µL 75 µM NBT and 500 µL 2 µM riboflavin as well as enzyme extracts (50–150 µL). The total volume was brought up to 5 mL with K-P (pH 7.6) containing 0.1 mM Na-EDTA. The reaction was started by adding the riboflavin to the mixture and placing the vials under the lights in growth chamber for about 8 min. One unit of SOD activity is defined as the SOD activity that results in a 50 % decrease in the NBT reduction. Glutathione reductase activity was determined by recording the oxidation of nicotinamide adenine dinucleotide phosphate (NADPH) at 340 nm according to Foyer and Halliwell (1976) with a few modifications. The 1-mL reaction mixture consisted of 100 µL of 0,5 mM oxidized glutathione (GSSG), 100 µL of 0.12 mM NADPH, 50–150 µL of the enzyme extract and 650–750 µL of 50 mM K-P buffer (pH 7.6) with 0.1 mM Na-EDTA. Results were adjusted for the non-enzymatic oxidation of NADPH by observing the decrease of absorbance at 340 nm in the absence of GSSG. Ascorbate peroxidase activity was measured according to Nakano and Asada (1981) by monitoring the decrease in absorbance of ascorbic acid at 290 nm. The 1-ml reaction mixture contained, 100 µL of 12 mM H_2O_2, 100 µL of 0.25mM ascorbic acid, 50–150 µL of the enzyme extract in addition to 650–750 µL of 50mMK-P buffer (pH 7.6) containing 0.1 mM Na-EDTA. Catalase activity was determined by monitoring the decrease in the absorbance of H_2O_2 at 240 nm. The reaction mixture contained 100 µL of 100 mM H_2O_2 dissolved in K-P buffer, 50–

150 µL of the enzyme extract and sufficient 50 mM K-P buffer (pH 7.6) containing 0.1 mM Na-EDTA to bring up the total volume to 1 mL.

3. Results and Discussion

Germination of spelt cultivars was significantly affected by the salt stress and morphologically it was quite easy to choose which ones were the best candidates to this stress. For the first experiment, the best one was Sp912. The worst was Sp804 in salt stress conditions.
Then, the efficiency was calculated by dividing the stress dry matter, by the respective control's dry matter.

These results are showed in the follow charts (Fig. 3.1):

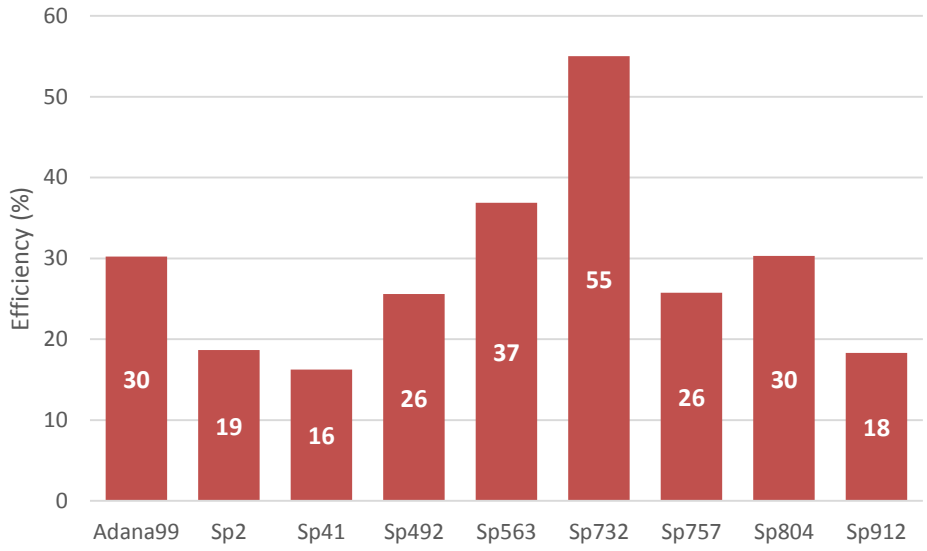

Fig. 3.1 - Salt efficiency from first experiment.

These results shows that the genotypes Sp732 (55%) and Sp563 (37%), have better efficiency when compared with the reference genotype. However only these results doesn't prove that the previously spelt genotypes hold some traits that confer resistance to salt stress.
Then the mineral content was determined by ICP (for data details see Appendix 1). Nutrient disturbances under salinity reduce plant growth by affecting the availability, transport, and partitioning of nutrients but also an increased nutrient supply will not improve plant growth when the nutrient is already present in sufficient amounts in the soil and when the salt stress is severe (Hu and Schmidhalter, 2005). It is important analyze what salt causes in terms of mineral nutrients. In this way, another concern is that the nutrient content of the soil should be sufficient such that addition of salt does not cause nutrient deficiency by decreasing the activity of other ions (Verslues *et al*., 2006). In the first table of Appendix 1 dry matter results,

efficiency data and the concentrations of the most important minerals for the study of salt stress (K^+, Ca^{2+} and Na^+) are shown. It is the interplay of these ions, which brings homeostasis in the cell (Mahajan and Tuteja, 2005). Potassium is an essential factor in protein synthesis, glycolytic enzymes, and photosynthesis; an osmotic mediating cell expansion and turgor-driven movements; and a competitor of Na^+ under saline conditions. High Na^+ concentrations in the external solution cause a decrease in both K^+ and Ca^{2+} concentrations in the tissues of many plant species (Hu and Schmidhalter, 2005). So, after determination of mineral content, the ratio K/Na was calculated (Fig. 3.2; Fig. 3.3). Because under salt stress K/Na ratio is too different between control and stress condition, charts were drawn separately:

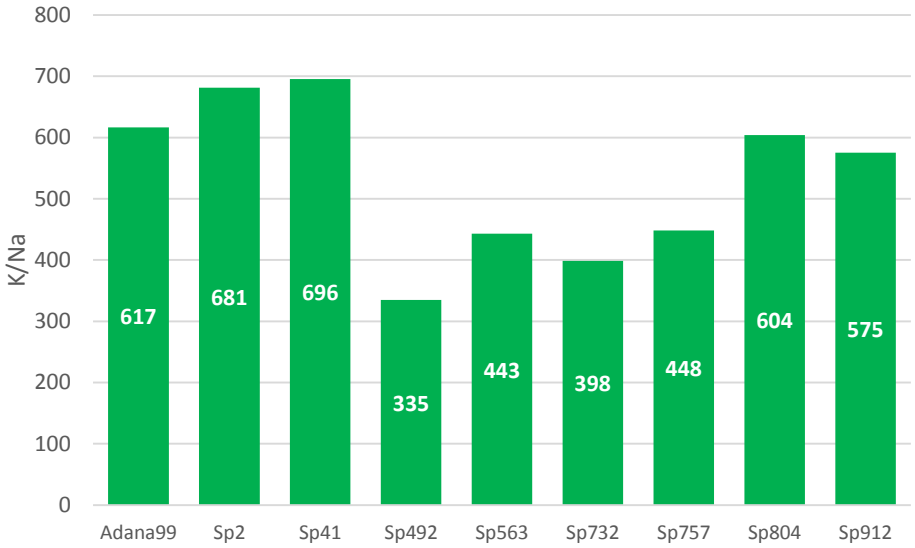

Fig. 3.2 - K/Na ratio from all genotypes tested from the first experience under control conditions.

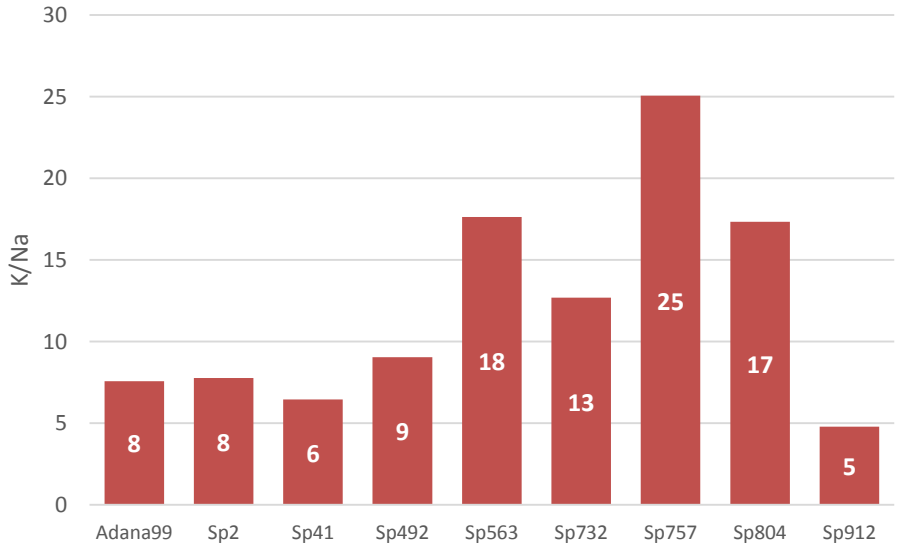

Fig. 3.3 - K/Na ratio from all genotypes tested from the first experience under salt stress conditions.

The response of the genotypes tested is clearly different when submitted to stress. In control conditions, the genotype Sp2, Sp41 and Sp912 have a better K/Na ratio than reference genotype, but when submitted to salt stress these three have the lowest ratio, on the other hand, the remaining genotypes have higher ratio than reference genotype. This results prove that most of spelt genotypes used in this experiment have better K/Na ratio and possibly the genotypes Sp563, Sp732 and Sp804 have better growth efficiency in salt stress conditions.

A high cytosolic K/Na ratio is very important because intracellular K^+ and Na^+ homeostasis is important for the activities of many cytosolic enzymes, and for maintaining membrane potential and an appropriate osmotic for cell volume regulation (Zhu, 2003). The results shows that Na^+ contents increased under saline condition, and this increase results in a decrease of K/Na ratio in all genotypes studied (Fig. 3.2; Fig. 3.3).

In 2003, Zhu described that Na^+ stress disrupts K^+ uptake by root cells, and when Na^+ enter in the cells and accumulates to high levels, it becomes toxic to enzymes. To prevent growth cessation or cell death, excessive Na^+ has to be expelled or compartmentalized in the vacuole (Zhu, 2003). Plant vacuoles constitute 40–90% of the total intracellular volume of a mature plant cell and, in concert with the cytosol, generate the cell turgor responsible for growth and plant rigidity (Gaxiola *et al*., 2001). This compartmentation system not only lowers Na^+ concentration in the cytoplasm but also contributes to osmotic adjustment to maintain water uptake from saline solutions (Zhu, 2003), which means that increased vacuolar solute accumulation could confer salt tolerance (Gaxiola *et al*., 2001). Other organelles, such as plastids and mitochondria, may also accumulate some Na^+ (Zhu, 2003).

Plants limiting the uptake of toxic ions or maintaining normal nutrient ion contents could show greater tolerance (Khan, *et al*., 2009) which is the case of some genotypes already mentioned in the present study.

The capacity to store Na^+ in vacuoles can explain why the genotypes Sp563, Sp732 and Sp804 have better salt efficiency than Sp757 even having lower K/Na ratio (Fig. 3.2; Fig. 3.3), because if the capacity of the plant to store is very high, its metabolic activity is not so affected by Na^+ toxicity, however when we milling, all salt inside the vacuoles will be released and mixed in the sample. However, due to problems of seed germination in this experiment, it is not possible to state with certainty that the results reflect reality. For this reason in the second experiment all seeds were vernalized before sowing. Other genotypes were selected for this experiment to extend our range of genotypes. We also concluded that 2500ppm of salt had a severe effect on seed germination and on plant's development, then we decided reduce for 2000ppm on the future experiments.

In the second experiment, seed germination was successful. Yet a high rate of germination under salt stress is not well correlated with salinity tolerance at later developmental stages (Verslues *et al*., 2006). This time, morphologically, the best two genotypes were Sp92 and Sp96 for salt stress. The worst were Sp53 and Sp801 for salt stress.

As in the first experiment, the efficiency was calculated and it's shown below (Fig. 3.4):

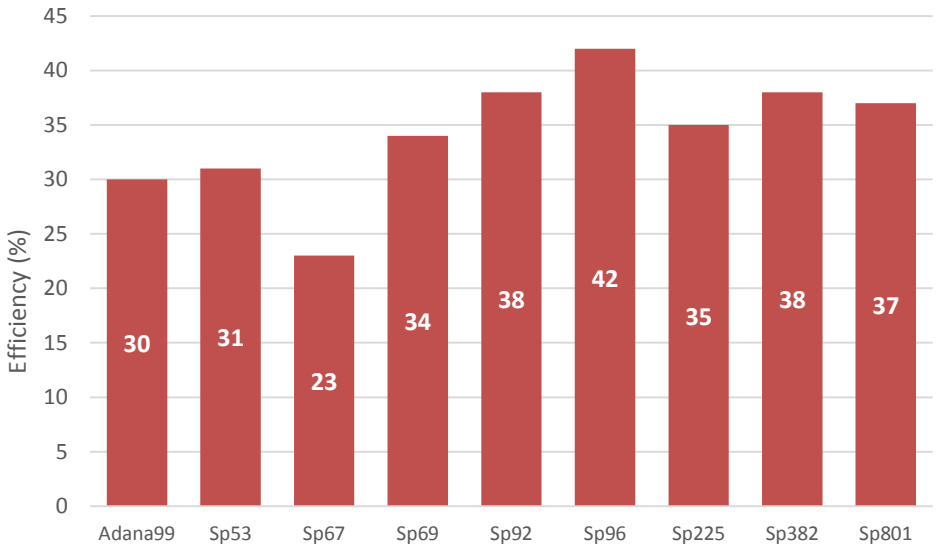

Fig. 3.4 - Salt efficiency from second experiment.

This time, the genotypes Sp53 (31%), Sp69 (34%), Sp92 (38%), Sp96 (42%), Sp225 (35%), Sp382 (38%) and Sp801 (37%) had better efficiency when compared with the reference genotype for salt stress.

Again, after determination of mineral content (Appendix 2), the ratio K/Na was calculated and it is shown on the next two figures (Fig. 3.5 and Fig. 3.6):

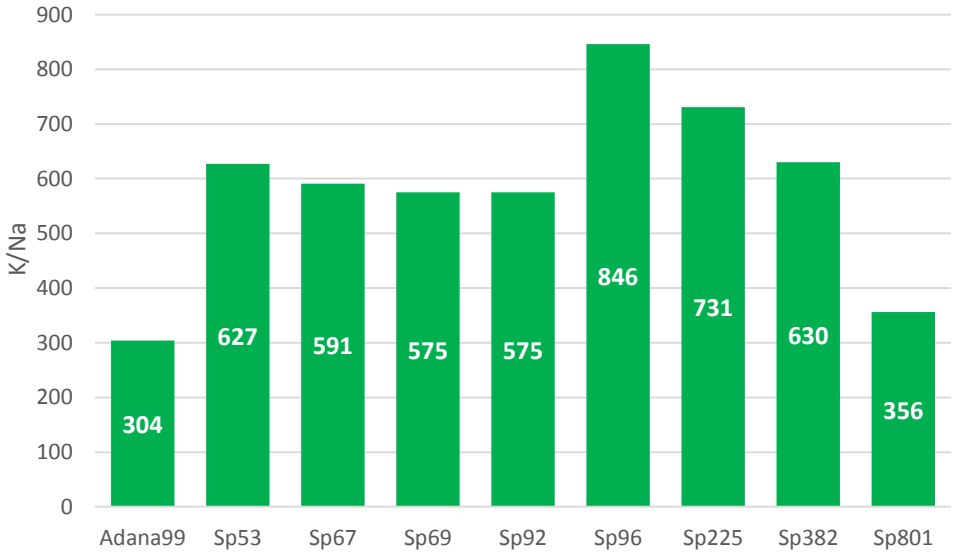

Fig. 3.5 - K/Na ratio from all genotypes tested from the second experience under control conditions.

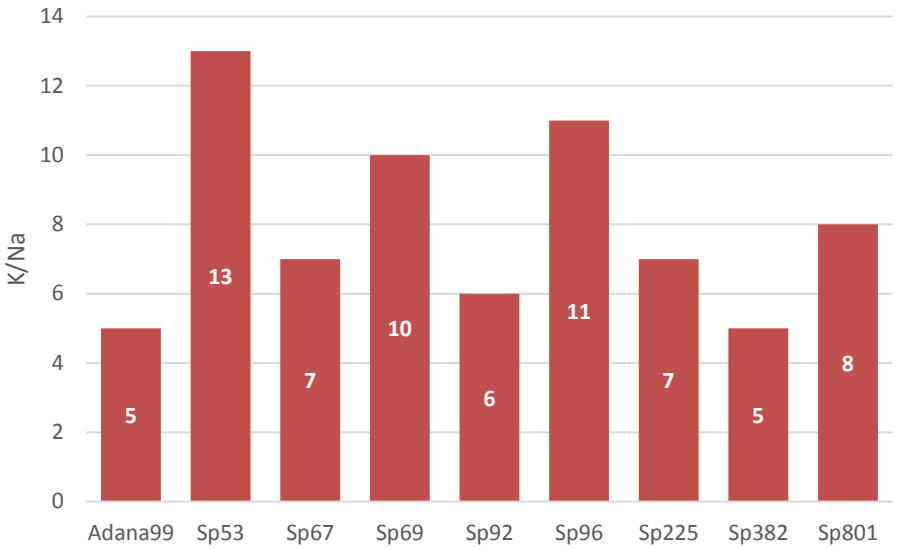

Fig. 3.6 - K/Na ratio from all genotypes tested from the second experience under salt stress conditions.

As in the first experiment, a negative correlation in K/Na ratio was observed between the control and salt stress genotypes studied (Fig. 3.5 and Fig. 3.6). In both control and salt stress conditions, all genotypes studied have higher K/Na ratio than modern wheat and, excluding the genotype Sp67, which have lower biomass yield (salt efficiency) than modern wheat, all of them are probably more resistant to salt stress conditions than modern wheat.

While table 1 and 3 from Appendix 1 and 2, respectively, shows the concentrations of the most important minerals for this study, table 2 and 4 (from Appendix 1 and 2, respectively) reveals the concentrations of P, S, Mg and some micronutrients (Zn, Fe, Mn, Cu, B and Al). The availability of micronutrients in saline soils depends on the solubility of the micronutrients, the pH and pE of the soil solution, and the nature of the binding sites on the organic and inorganic-particle surfaces. Thus, salinity can differentially affect the micronutrient concentrations in the plant, depending upon the crop species and the salinity level (Hu and Schmidhalter, 2005). In case of spelt the results in terms of micronutrients were different between the first and second experiment. In the first experiment (table 2, Appendix 1) was verified a significant increase of almost all micronutrients when under salt stress (except for B), while for the second experiment (table 4, Appendix 2) this general increase does not occurred. At nutritional level the results from first experiment were very good, once shows that spelt genotypes had more capacity for absorb the micronutrients than the reference (Adana99), which showed a decrease in almost all micronutrients (except for Zn) when compared with control condition. In second experiment the micronutrients % does not increase from control condition to salt stress conditions, but does not decrease, was not observed a significant difference between control and salt stress conditions. These results are also good once that high salt deposition in

the soil generate a low water potential zone in the soil making it increasingly difficult for the plant to acquire both water as well as nutrients (Mahajan and Tuteja, 2005).

For better evaluation of our results, the efficiency vs K/Na chart was drawn for first (Fig. 3.7) and second (Fig. 3.8) experiment:

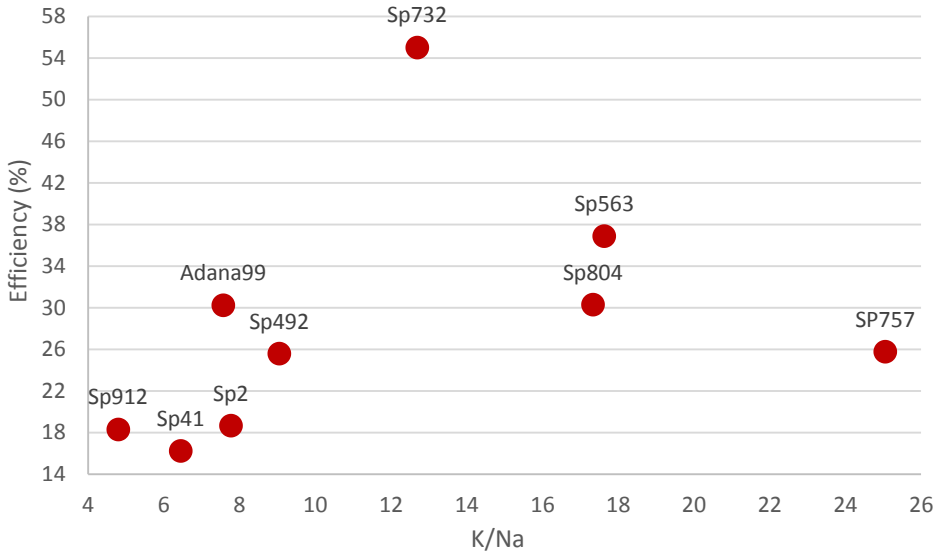

Fig. 3.7 - Efficiency vs K/Na from the results from the first experiment for salt stress conditions.

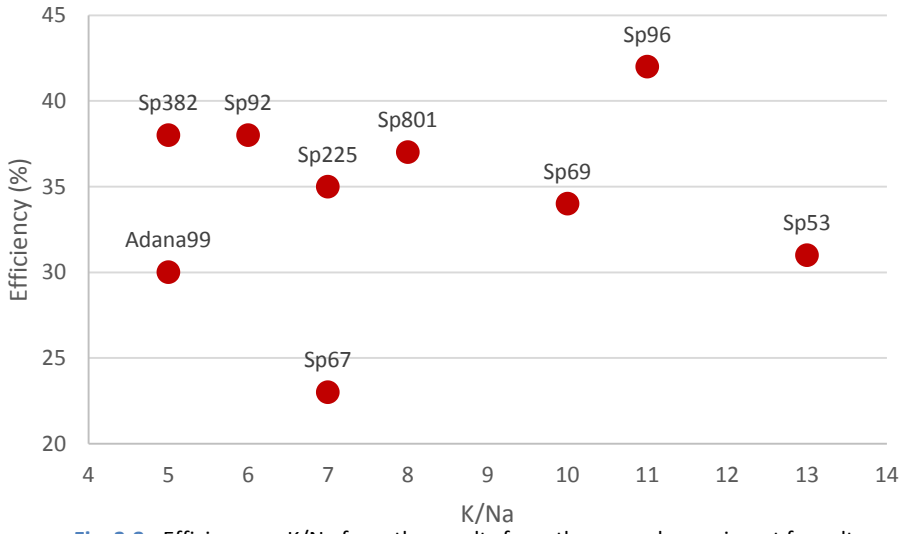

Fig. 3.8 - Efficiency vs K/Na from the results from the second experiment for salt stress conditions.

The previous charts allow a conclusion about which genotypes could come to be more resistant under salt stress. Using this information we can say that from the first experiment genotypes Sp563, Sp732 and Sp757 are good candidates for salt stress. From the second experiment the genotypes Sp69, Sp96 and Sp53 for salt stress are good candidates for these stresses.

For the third experiment, we select the best and worst genotypes for both salt tolerance from the previous experiments. The genotypes selected as good for salt tolerance were the Sp69, Sp92, Sp96, Sp563 and Sp732 and the bad ones were the Sp41, Sp67 and Sp912.

Unfortunately the genotypes Sp53 and Sp757, which were the better candidates as it can be seen in our results, it could not be selected for this experiment due to the lack of stock of seeds in our bank of seeds.

In the next two figures (Fig. 3.9; Fig. 3.10) it is possible see the morphological aspect of the two worst and two best genotypes from the 3rd experiment:

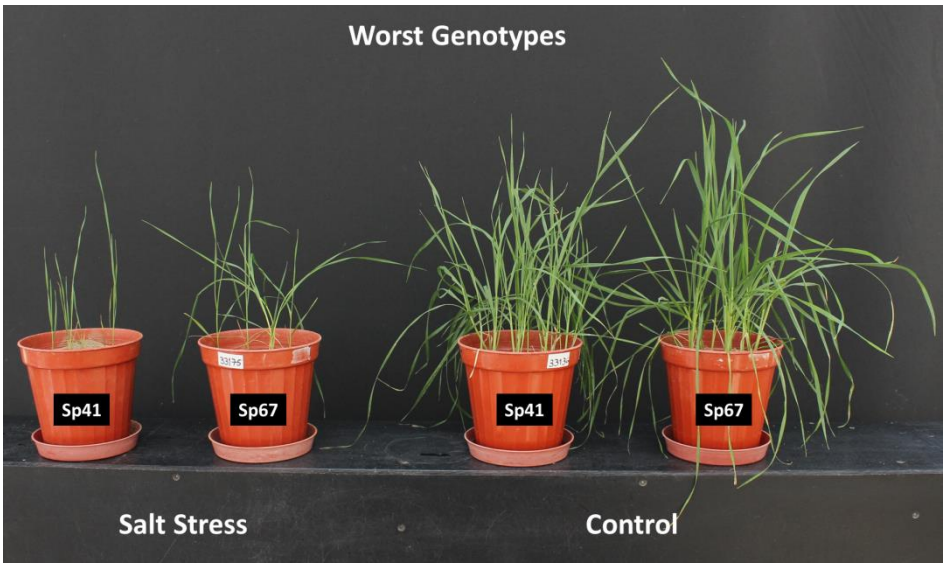

Fig. 3.9 - Worst Genotypes in salt stress conditions with respective controls.

For the worst genotype was chosen the genotype Sp41, and for second worst the genotype Sp67 as it can be seen in Fig. 3.9.

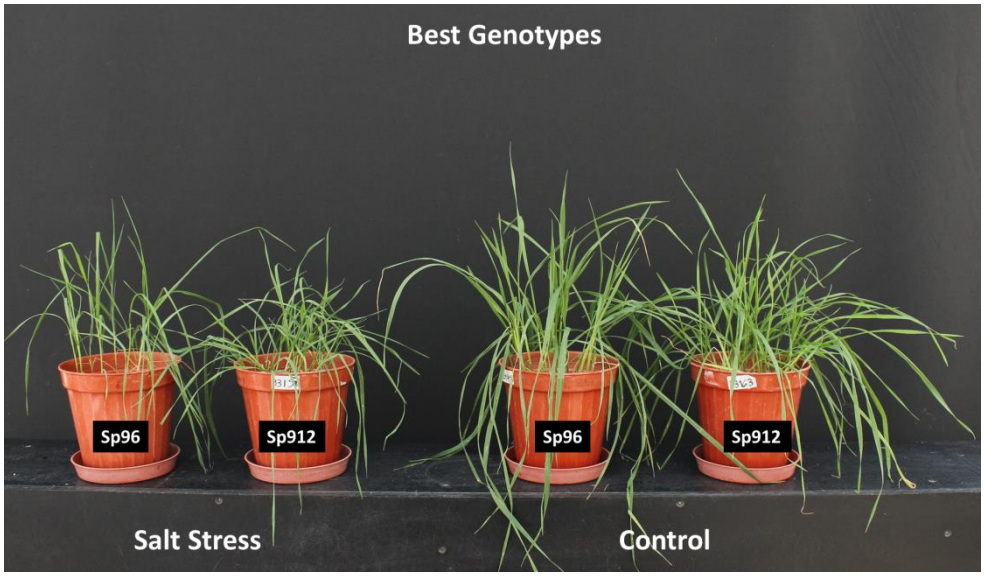

Fig. 3.10 - Best Genotypes in salt stress conditions with respective controls.

For the best genotype were chosen two genotypes: Sp96 and Sp912 as it can be seen in Fig. 3.10. In Fig. 3.11 it is possible compare the two best with the two worst genotypes

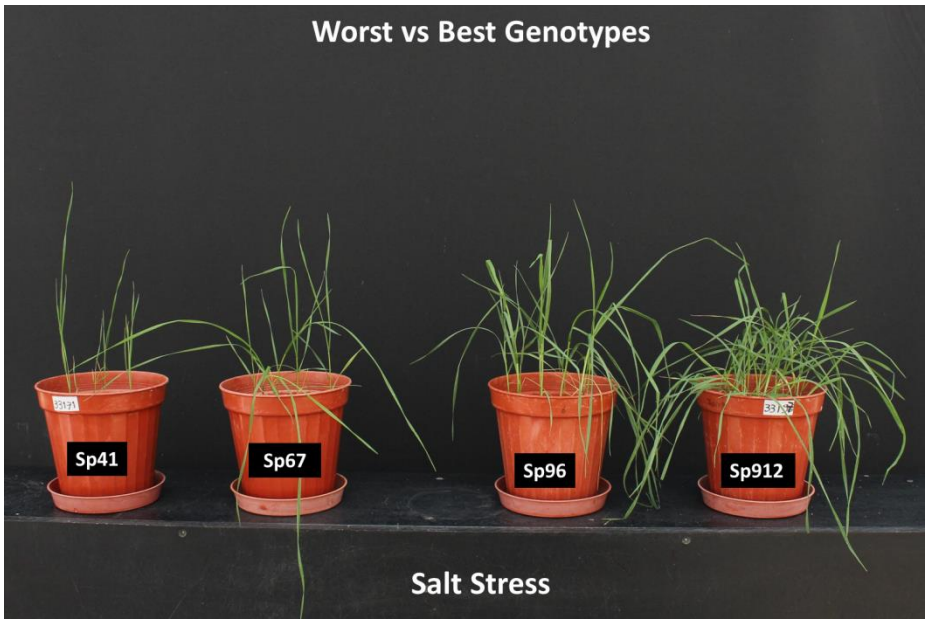

Fig. 3.11 - Worst Genotypes vs Best Genotypes in salt stress conditions.

In the previous figures we can realize that under salt stress the leaves become darker and thinner when compared with the control, especially for the two worst genotypes (Fig. 3.9). For the two best genotypes the leaves are still thin compared with control, but the differences in color are not so notable (Fig. 3.10). In terms of biomass production is clearly that occurred a significant reduction under salt stress, especially in genotype Sp41 and Sp67.

In relation to efficiency the worst genotype was the genotype Sp41 with 15% of efficiency, which is according to morphologically analysis. The best genotype is also according with morphologically analysis – genotype Sp912 with 49% efficiency – as it can be seen in the following graph (Fig. 3.12).

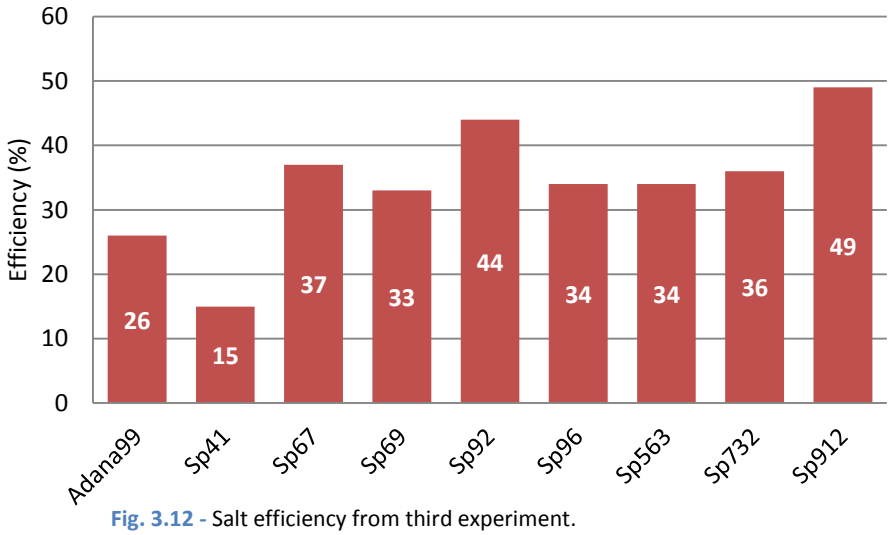

Fig. 3.12 - Salt efficiency from third experiment.

For those two genotypes (the best and the worst) it is verified a significant difference comparing with the others. In terms of efficiency the second bad spelt genotype was Sp69 and the second good one was genotype Sp92, contradicting the morphological analysis results.

The mineral content from third experiment was determined (Appendix 3), and was obtained a good result at nutritional level once again. After that the ratio K/Na was calculated and it is shown on the next two figures (Fig. 3.13; Fig. 3.14):

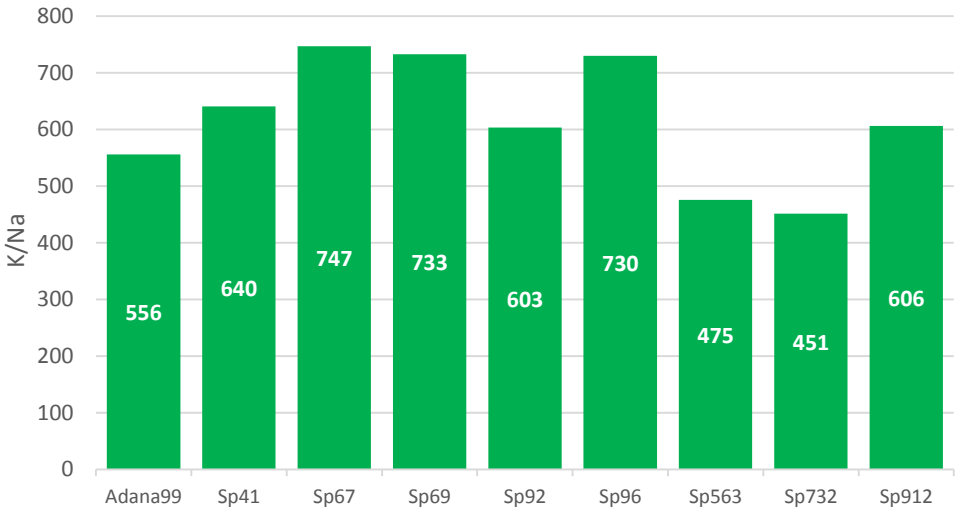

Fig. 3.13 - K/Na ratio from all genotypes tested from the third experience under control conditions.

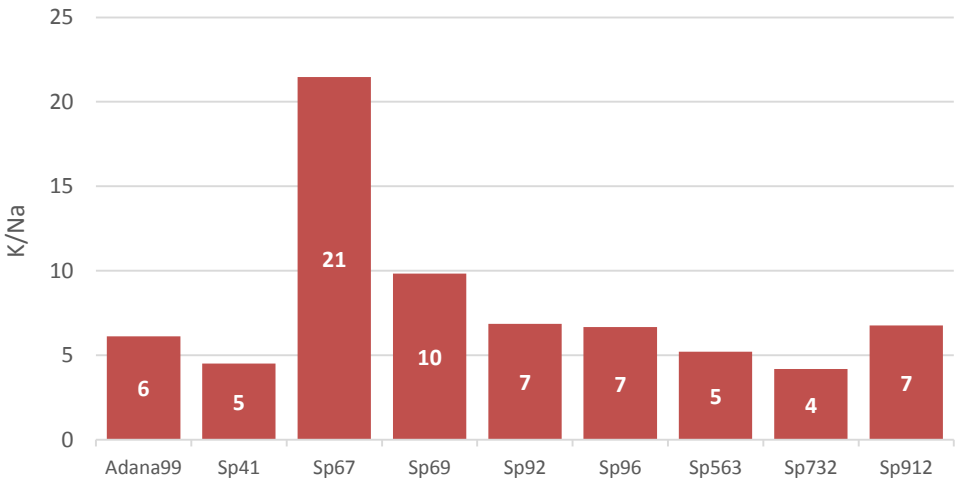

Fig. 3.14 - - K/Na ratio from all genotypes tested from the third experience under salt stress conditions

As was expected the ratio K/Na had a significant decrease when under salt stress conditions, what already occurred in the previous experiments. The K/Na ratio from genotypes Sp41, Sp92 Sp732 is too low (6). In case of genotype Sp41 this fact was expected and this result reinforces what was already noticed from efficiency results, morphological results and also from first experiment (Fig. 3.3) that genotype, Sp41, is a bad genotype in salt stress conditions. In case of genotype Sp92 the K/Na ratio from third experiment is the same from the second experiment

(Fig. 3.6), what confirms that Sp92 does not have a good mechanism of Na^+ exclusion from leaf blades, since his Na^+ content in the plant should be very high which causes a high K/Na ratio. However Sp92 efficiency`s (Fig. 3.12) was the second highest and was also high on second experiment (Fig. 3.4), which means that his capacity to store Na^+ in vacuoles should works very well, in this way its metabolic activity is not so affected by Na^+ toxicity. However when we milling, all salt inside the vacuoles will be released and mixed in the sample, and the K/Na ratio obtained is high. For Sp732 genotype third experiment K/Na ratio results are not according with the previous experiment (Fig. 3.3), so, we can´t conclude anything about this genotype. This time genotype Sp67 had the highest K/Na ratio what was not expected based on morphologic results and second experiment results (Fig. 3.6). Zhu *et al*., (2001) found that plants are able to tolerate moderately saline environments with a greater ability to exclude Na^+ from shoot or at least the leaf blade and concurrently maintain high level of K^+. Similarly the K^+ content in shoots is an index of osmotic adjustment. Tammam *et al*. (2008) records that high K/Na ratio is more important for many species than simply maintaining a low concentration of Na^+.

For third experiment the chart of Efficiency vs K/Na (Fig. 3.15) was also drawn:

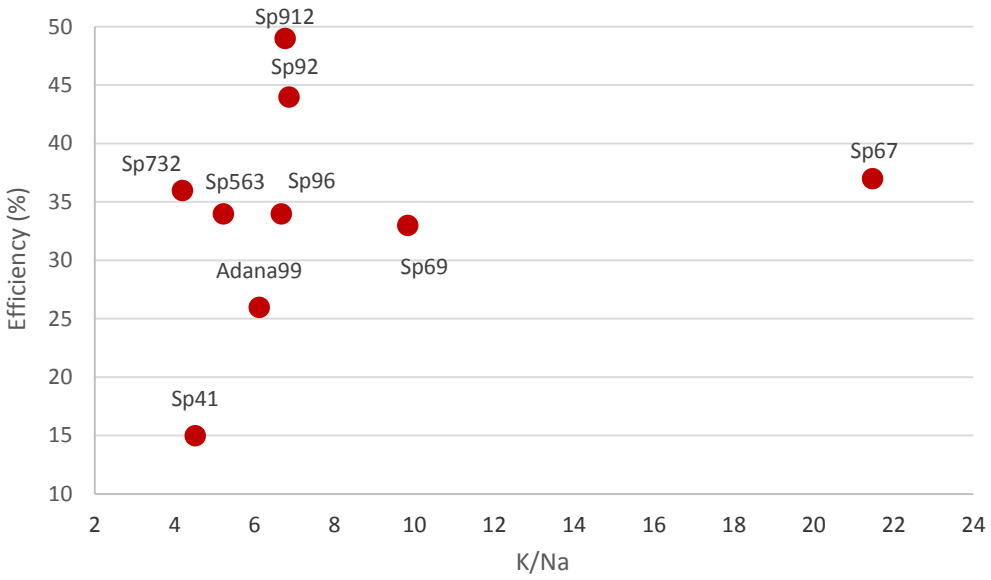

Fig. 3.15 - Efficiency vs K/Na from the results from the first experiment for salt stress conditions

Looking to this chart (Fig. 3.15) is clearly that genotype Sp41 is not a salt tolerant genotype as already has been showed. Based on this chart the genotype Sp912, Sp69 and Sp67 are the best candidates for salt tolerant genotypes.

One approach to understanding the ROS-scavenging systems in plant stress tolerance is to manipulate the levels of antioxidant enzyme activities (Yousuf *et al.*, 2012) , so this time it was determined the enzymatic activity of the plant in genotypes Sp41, Sp67, Sp92, Sp96 and Sp912 (appendix 4). It was calculated the protein concentration, SOD Activity, GR Activity, CAT Activity and APX Activity. In general protein concentration had a slight increase under salt stress conditions (except genotype Sp41). It is expected that under salt stress conditions occurs fragmentation of proteins due to toxic effects of reactive oxygen species which results in a reduction of protein content and also activity of protease or other catabolic enzymes results in protein degradation (Mafakheri *et al*., 2011). Nevertheless Tammam *et al*. (2008) relates that the increase in soluble protein in shoots might indicate the superiority of shoots over roots to alleviate the imposed salt stress. As well, Datta *et al*. (2009) reports an increase in protein content of leaves in the salt concentrations as compared with the control. Also, to fight against ROS, plants produce various proteins, as enzymes. As we can see in Appendix 4 almost all production of enzymes increases under salt stress conditions (except production of CAT and GR for genotypes Sp41 and Sp67). The results show as genotypes Sp92, Sp96 and Sp912 have a better mechanism to combat reactive oxygen species than genotypes Sp41 and Sp67, once these genotypes (Sp92, Sp96 and Sp912) can produce more antioxidant enzymes. Minimization of reactive oxygen species as a result of inhibition of photosynthesis and maximization of their removal (scavenging) is likely to be an important response to high salinity (Zhu *et al*., 2001).

Conclusions and Future Prospects

Despite the fact that research efforts have produced an enormous amount of information, it is difficult to asserting with certainty which are the salt sensitive or tolerant genotypes. Only a few components have been the subject in this study, being obtained some contradictory results.

This study on effect of salt in several Spelt Wheat genotypes showed that first of all salinity causes a significant reduction on plant shoot length. High level of salinity may have also inhibit the root and shoot elongation due to slowing down the water uptake for overall osmotic adjustments of the plant under high salt stress condition. In this study was found that genotype Sp41 is a salt sensitive genotype as seen in the data about morphological analyses, as well as in terms of efficiency and low K/Na ratio. Based on second experiment and on morphological analysis from third experiment was expected that genotype Sp67 were also a salt sensitive genotype, however in third experiment this genotype obtained a high K/Na ratio, and a high K/Na ratio is more important for many species than simply maintaining a low concentration of Na^+, consequently a high K/Na ratio is an indicator of salt tolerance. Based on this inconsistent result more studies may be focused on this genotype. Also in stress tolerance the results remains controversial, once that in third experiment the genotype Sp912 has revealed as a salt tolerant genotype, but the results were different in first experiment. The genotypes Sp69 and Sp96 are good candidates for salt tolerance, once shows consistent results in the two experiments. A good result obtained in this study were that almost all Spelt Wheat genotypes (except Sp41) get better results than the Wheat genotype used as reference, Adana99. Thus, it is concluded that Spelt crops are more resistance than Bread Wheat crops under salt stress conditions. Future works should be focused in study more parameters (for example: carbohydrates content, transpiration rate, membrane integration, photosynthetic pigments) to get more consistent data. Also, it is crucial continue to study Spelt genotypes in order to find resistant genotypes to be used in breeding programs, once that agricultural production has become gradually more intense which leads to soil's degradation.

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Appendix

Appendix 1 - 1º Experiment

Table. 1 - Mineral composition of K, Ca and Na from genotypes studied from first experiment, as well as, salt efficiency and K/Na and (K+Ca)/Na ratios with respective standard deviations (STD).

	Genotype	Dry matter (g.plant^{-1})	Salt Efficiency (%)	K (%)	Ca (%)	Na (%)	K/Na	(K+Ca)/Na
Control	Sp732	1.23 ± 0.09	-	7.402 ± 1.237	0.752 ± 0.075	0.019 ± 0.002	398	439
	Sp563	1.14 ± 0.03	-	8.493 ± 0.401	0.796 ± 0.023	0.019 ± 0.001	443	485
	Sp804	1.45 ± 0.25	-	7.076 ± 0.212	0.572 ± 0.024	0.012 ± 0.001	604	653
	Sp757	1.37 ± 0.13	-	7.860 ± 0.264	0.698 ± 0.038	0.018 ± 0.001	448	488
	Sp492	1.15 ± 0.04	-	7.069 ± 1.709	0.703 ± 0.076	0.021 ± 0.003	335	368
	Sp2	1.30 ± 0.07	-	7.576 ± 0.496	0.640 ± 0.008	0.011 ± 0.001	681	739
	Sp912	1.10 ± 0.04	-	6.423 ± 0.181	0.636 ± 0.072	0.011 ± 0.002	575	632
	Sp41	1.26 ± 0.10	-	6.446 ± 0.940	0.589 ± 0.053	0.009 ± 0.002	696	759
	Ada99	0.90 ± 0.01	-	6.630 ± 0.275	0.727 ± 0.025	0.011 ± 0.001	617	684
2500ppm NaCl	Sp732	0.68	55	7.116	0.840	0.561	13	14
	Sp563	0.42	37	6.954	0.783	0.394	18	20
	Sp804	0.44	30	7.596	0.782	0.438	17	19
	Sp757	0.35 ± 0.22	26	6.673 ± 0.636	0.869 ± 0.088	0.266 ± 0.065	25	28
	Sp492	0.29 ± 0.06	26	7.431 ± 0.138	1.042 ± 0.016	0.821 ± 0.089	9	10
	Sp2	0.24 ± 0.04	19	6.390 ± 0.593	1.115 ± 0.256	0.822 ± 0.154	8	9
	Sp912	0.20 ± 0.02	18	6.137 ± 0.451	1.288 ± 0.178	1.280 ± 0.075	5	6
	Sp41	0.21 ± 0.02	16	6.094 ± 0.851	1.326 ± 0.171	0.945 ± 0.225	6	8
	Ada99	0.27 ± 0.08	30	4.543 ± 0.374	0.967 ± 0.142	0.600 ± 0.107	8	9

The data shown are approximations. The calculations were done with the real values.

Table. 2 - Mineral composition from genotypes studied in the first experiment, with respective STD.

	Genotype	P	Dry matter (g.plant^{-1})	S	Salt Efficiency (%)	Mg	K (%)	Zn	Ca (%)	Fe	Na (%)	Mn (mg.kg^{-1})	Cu	K/Na	B	(K+Ca)/Na	Al
Control	Sp732	0.444 ±.020	1.23 ± 0.09	0.386 ±0.036	-	0.203 ±0.015	7.402 ± 1.237	50 ± 0	0.752 ± 0.075	46 ± 1	0.019 ± 0.002	122 ± 5	9 ± 1	398	16 ± 1	439	8 ± 0
	Sp563	0.509 ± 024	1.14 ± 0.03	0.420 ±0.020	-	0.215 ±0.004	8.493 ± 0.401	59 ± 6	0.796 ± 0.023	47 ± 2	0.019 ± 0.001	116 ± 9	9 ± 0	443	15 ± 2	485	8 ± 1
	Sp804	0.467 ±0.061	1.45 ± 0.25	0.360 ±0.034	-	0.183 ±0.011	7.076 ± 0.212	50 ± 3	0.572 ± 0.024	50 ± 1	0.012 ± 0.001	93 ± 7	8 ± 0	604	16 ± 1	653	8 ± 0
	Sp757	0.492 ±0.015	1.37 ± 0.13	0.354 ±0.007	-	0.189 ±0.010	7.860 ± 0.264	46 ± 1	0.698 ± 0.038	47 ± 4	0.018 ± 0.001	115 ± 4	9 ± 0	448	15 ± 2	488	7 ± 2
	Sp492	0.416 ±0.031	1.15 ± 0.04	0.334 ±0.022	-	0.173 ±0.023	7.069 ± 1.709	47 ± 5	0.703 ± 0.076	42 ± 5	0.021 ± 0.003	115 ± 6	8 ± 1	335	15 ± 2	368	8 ± 2
	Sp2	0.450 ±0.023	1.30 ± 0.07	0.359 ±0.010	-	0.186 ±0.017	7.576 ± 0.496	56 ± 3	0.640 ± 0.008	53 ± 2	0.011 ± 0.001	113 ± 14	9 ± 1	681	16 ± 1	739	13 ± 0
	Sp912	0.471 ±0.055	1.10 ± 0.04	0.373 ±0.035	-	0.175 ±0.017	6.423 ± 0.181	51 ± 6	0.636 ± 0.072	54 ± 3	0.011 ± 0.002	109 ± 4	8 ± 0	575	19 ± 2	632	11 ± 3
	Sp41	0.421 ±0.026	1.26 ± 0.10	0.332 ±0.021	-	0.166 ±0.018	6.446 ± 0.940	49 ± 3	0.589 ± 0.053	44 ± 8	0.009 ± 0.002	123 ± 5	8 ± 1	696	14 ± 1	759	4 ± 0
	Ada99	0.448 ±0.010	0.90 ± 0.01	0.369 ±0.014	-	0.178 ±0.008	6.630 ± 0.275	55 ± 7	0.727 ± 0.025	50 ± 1	0.011 ± 0.001	145 ± 2	9 ± 1	617	16 ± 1	684	14 ± 3
2500ppm NaCl	Sp732	0.486	0,68 ± -	0.311	55	0.242	7.116 ± -	84	0,840 ± -	52	0.561 ± -	177	11	13	11	14	6
	Sp563	0.554	0,42 ± -	0.326	37	0.250	6.954 ± -	84	0,783 ± -	65	0.394 ± -	167	11	18	11	20	14
	Sp804	0.489	0,44 ± -	0.316	30	0.231	7.596 ± -	96	0,782 ± -	58	0.438 ± -	186	11	17	11	19	7
	Sp757	0.466 ±0.067	0,35 ± 0,22	0.341 ±0.028	26	0.217 ±0.023	6.673 ± 0.636	55 ± 9	0.869 ± 0.088	59 ± 3	0.266 ± 0.065	175 ± 12	11 ± 1	25	13 ± 0	28	12 ± 4
	Sp492	0.463 ±0.055	0,29 ± 0,06	0.332 ±0.003	26	0.232 ±0.012	7.431 ± 0.138	66 ± 9	1.042 ± 0.016	62 ± 5	0.821 ± 0.089	188 ± 4	11 ± 0	9	14 ± 0	10	16 ± 9
	Sp2	0.389 ±0.063	0,24 ± 0,04	0.307 ±0.022	19	0.223 ±0.023	6.390 ± 0.593	60 ± 7	1.115 ± 0.256	66 ± 14	0.822 ± 0.154	158 ± 17	9 ± 0	8	12 ± 2	9	31 ± 13
	Sp912	0.467 ±0.091	0,20 ± 0,02	0.321 ±0.027	18	0.217 ±0.024	6.137 ± 0.451	79 ± 10	1.288 ± 0.178	59 ± 2	1.280 ± 0.075	158 ± 20	10 ± 1	5	11 ± 1	6	16 ± 5
	Sp41	0.445 ±0.065	0,21 ± 0,02	0.331 ±0.009	16	0.221 ±0.002	6.094 ± 0.851	48 ± 8	1.326 ± 0.171	71 ± 13	0.945 ± 0.225	164 ± 39	11 ± 1	6	13 ± 1	8	24 ± 14
	Ada99	0.436 ±0.044	0,27 ± 0,08	0.251 ±0.020	30	0.178 ±0.011	4.543 ± 0.374	57 ± 11	0.967 ± 0.142	42 ± 4	0.600 ± 0.107	141 ± 16	8 ± 0	8	11 ± 1	9	5 ± 0

Appendix 2 - 2º Experiment

Table. 3 - Mineral composition of K, Ca and Na from genotypes studied from first experiment, as well as, salt efficiency and K/Na and (K+Ca)/Na ratios with respective STD.

	Genotype	Dry matter (g.plant^{-1})	Salt Efficiency (%)	K (%)	Ca (%)	Na (%)	K/Na	(K+Ca)/Na
Control	Sp96	0.40 ± 0.01	-	5.446 ± 0.222	0.705 ± 0.012	0.006 ± 0.000	846	955
	Sp92	0.46 ± 0.03	-	5.177 ± 0.075	0.695 ± 0.013	0.009 ± 0.001	575	652
	Sp382	0.40 ± 0.06	-	5.241 ± 0.061	0.730 ± 0.021	0.008 ± 0.001	630	718
	Sp801	0.35 ± 0.05	-	4.905 ± 0.144	0.764 ± 0.008	0.014 ± 0.001	356	411
	Sp225	0.43 ± 0.06	-	5.283 ± 0.197	0.653 ± 0.022	0.007 ± 0.002	731	822
	Sp69	0.43 ± 0.04	-	5.049 ± 0.133	0.724 ± 0.025	0.009 ± 0.001	575	657
	Sp53	0.33 ± 0.02	-	5.091 ± 0.121	0.805 ± 0.008	0.008 ± 0.002	627	726
	Sp67	0.37 ± 0.01	-	5.040 ± 0.112	0.625 ± 0.033	0.009 ± 0.001	591	664
	Ada99	0.49 ± 0.01	-	4.535 ± 0.163	0.583 ± 0.021	0.015 ± 0.002	304	343
2000ppm NaCl	Sp96	0.17	42	4.653 ± 0.193	0.887 ± 0.035	0.425 ± 0.068	11	13
	Sp92	0.17	38	4.118 ± 0.275	0.971 ± 0.037	0.686 ± 0.065	6	7
	Sp382	0.15	38	4.156 ± 0.121	1.024 ± 0.140	0.814 ± 0.090	5	6
	Sp801	0.13	37	4.207 ± 0.132	1.100 ± 0.069	0.529 ± 0.071	8	10
	Sp225	0.15	35	4.379 ± 0.145	1.011 ± 0.135	0.586 ± 0.028	7	9
	Sp69	0.15	34	4.262 ± 0.224	0.936 ± 0.061	0.444 ± 0.044	10	12
	Sp53	0.10	31	4.475 ± 0.201	0.916 ± 0.013	0.332 ± 0.038	13	16
	Sp67	0.09	23	4.250 ± 0.064	0.932 ± 0.076	0.649 ± 0.072	7	8
	Ada99	0.15	30	3.473 ± 0.121	0.838 ± 0.025	0.639 ± 0.119	5	7

The data shown are approximations. The calculations were done with the real values.

Table. 4 - Mineral composition from genotypes studied in the second experiment, with respective STD.

	Genotype	P	S (%)	Mg	Zn	Fe	Mn	Cu	B	Al
					(mg,kg^{-1})					
Control	Sp96	0,460 ±0,106	0,191 ± 0,018	0,408 ± 0,038	53 ± 11	60 ± 4	93 ± 12	11 ± 1	19 ± 4	6 ± 1
	Sp92	0,426 ±0,025	0,173 ± 0,002	0,410 ± 0,025	47 ± 3	53 ± 1	90 ± 6	9 ± 0	17 ± 2	4 ± 1
	Sp382	0,476 ±0,058	0,178 ± 0,021	0,393 ± 0,026	53 ± 10	61 ± 4	114 ± 20	11 ± 1	16 ± 2	4 ± 0
	Sp801	0,484 ±0,068	0,202 ± 0,017	0,428 ± 0,016	64 ± 8	61 ± 2	108 ± 11	11 ± 1	20 ± 1	5 ± 0
	Sp225	0,483 ±0,054	0,217 ± 0,012	0,489 ± 0,019	59 ± 6	66 ± 3	96 ± 8	11 ± 1	22 ± 2	7 ± 1
	Sp69	0,454 ±0,017	0,174 ± 0,011	0,393 ± 0,022	60 ± 8	62 ± 4	103 ± 11	11 ± 1	21 ± 2	6 ± 2
	Sp53	0,535 ±0,011	0,183 ± 0,009	0,390 ± 0,015	65 ± 8	61 ± 3	108 ± 9	11 ± 0	17 ± 2	5 ± 0
	Sp67	0,537 ±0,031	0,200 ± 0,009	0,385 ± 0,015	51 ± 5	59 ± 2	105 ± 8	11 ± 1	18 ± 1	4 ± 1
	Ada99	0,457 ±0,022	0,168 ± 0,006	0,408 ± 0,013	57 ± 4	51 ± 1	111 ± 9	9 ± 0	15 ± 2	7 ± 1
2000ppm NaCl	Sp96	0.472 ±0.066	0.193 ±0.004	0.352 ±0.004	38 ± 2	62 ± 1	90 ± 7	11 ± 0	16 ± 0	6 ± 1
	Sp92	0.450 ±0.028	0.182 ±0.010	0.344 ±0.014	40 ± 6	56 ± 3	95 ± 12	9 ± 0	17 ± 1	6 ± 1
	Sp382	0.517 ±0.064	0.180 ±0.016	0.352 ±0.012	45 ± 4	63 ± 4	100 ± 11	10 ± 1	16 ± 0	7 ± 1
	Sp801	0.504 ±0.088	0.208 ±0.017	0.375 ±0.024	43 ± 6	66 ± 4	111 ± 19	12 ± 1	17 ± 2	8 ± 1
	Sp225	0.478 ±0.017	0.224 ±0.004	0.388 ±0.022	43 ± 6	65 ± 1	105 ± 11	11 ± 0	18 ± 1	9 ± 3
	Sp69	0.448 ±0.051	0.178 ±0.016	0.344 ±0.017	42 ± 3	59 ± 5	86 ± 9	11 ± 1	15 ± 2	7 ± 1
	Sp53	0.408 ±0.017	0.198 ±0.022	0.333 ±0.027	39 ± 7	67 ± 6	91 ± 16	10 ± 2	17 ± 2	11 ± 5
	Sp67	0.557 ±0.037	0.193 ±0.005	0.348 ±0.007	36 ± 1	62 ± 1	86 ± 1	10 ± 0	16 ± 2	5 ± 1
	Ada99	0.455 ±0.085	0.170 ±0.008	0.335 ±0.018	40 ± 6	55 ± 6	90 ± 6	9 ± 1	15 ± 2	13 ± 5

Appendix 3 – 3º Experiment

Table. 5 - Mineral composition of K, Ca and Na from genotypes studied from first experiment, as well as, salt efficiency and K/Na and (K+Ca)/Na ratios with respective STD.

	Genotype	Dry matter (g.plant^{-1})	Salt Efficiency (%)	K (%)	Ca (%)	Na (%)	K/Na	(K+Ca)/Na
Control	S41	0.28 ± 0.03	-	5.103 ± 0.120	0.631 ± 0.031	0.008 ± 0.001	556	617
	S67	0.36 ± 0.09	-	5.290 ± 0.098	0.521 ± 0.035	0.007 ± 0.000	640	720
	Sp69	0.30 ± 0.05	-	5.241 ± 0.135	0.559 ± 0.039	0.007 ± 0.001	747	820
	Sp92	0.28 ± 0.03	-	5.053 ± 0.718	0.530 ± 0.014	0.008 ± 0.002	733	811
	Sp96	0.32 ± 0.02	-	5.460 ± 0.029	0.507 ± 0.025	0.007 ± 0.001	603	667
	Sp563	0.27 ± 0.03	-	5.282 ± 0.185	0.581 ± 0.042	0.011 ± 0.001	730	798
	Sp732	0.36 ± 0.11	-	4.790 ± 0.613	0.647 ± 0.121	0.011 ± 0.002	475	528
	Sp912	0.29 ± 0.03	-	5.524 ± 0.094	0.573 ± 0.025	0.009 ± 0.002	451	512
	Ada99	0.27 ± 0.06	-	4.653 ± 0.042	0.507 ± 0.038	0.008 ± 0.001	606	669
2000ppm NaCl	Sp41	0.04 ± 0.03	15	3.612	1.040	0.801	6	8
	Sp67	0.13 ± 0.10	37	4.406 ± 0.419	0.545 ± 0.116	0.205 ± 0.001	5	6
	Sp69	0.10 ± 0.04	33	4.403 ± 0.309	0.713 ± 0.083	0.448 ± 0.141	21	24
	Sp92	0.12 ± 0.05	44	3.968 ± 0.691	1.131 ± 0.420	0.579 ± 0.170	10	11
	Sp96	0.11 ± 0.01	34	4.233 ± 0.351	0.821 ± 0.067	0.635 ± 0.135	7	8
	Sp563	0.09 ± 0.04	34	3.890 ± 0.131	1.108 ± 0.069	0.746 ± 0.099	7	8
	Sp732	0.13 ± 0.03	36	3.755 ± 0.112	1.014 ± 0.080	0.898 ± 0.162	5	6
	Sp912	0.15 ± 0.04	49	4.350 ± 0.301	1.055 ± 0.082	0.644 ± 0.162	4	5
	Ada99	0.07 ± 0.03	26	3.288 ± 0.275	1.075 ± 0.272	0.538 ± 0.116	7	8

The data shown are approximations. The calculations were done with the real values.

Table. 6 - Mineral composition from genotypes studied in the third experiment, with respective STD.

	Genotype	Dry matter (g.plant^{-1})	Salt Efficiency (%)	K (%)	Ca (%)	Na (%)	K/Na	(K+Ca)/Na
Control	Sp732	1,23 ± 0,09	0.311 ±0.006	7,402 ± 1,237	0,752 ± 0,075	0,019 ± 0,002	398	439
	Sp563	1,14 ± 0,03	0.307 ±0.011	8,493 ± 0,401	0,796 ± 0,023	0,019 ± 0,001	443	485
	Sp804	1,45 ± 0,25	0.305 ±0.008	7,076 ± 0,212	0,572 ± 0,024	0,012 ± 0,001	604	653
	Sp757	1,37 ± 0,13	0.335 ±0.042	7,860 ± 0,264	0,698 ± 0,038	0,018 ± 0,001	448	488
	Sp492	1,15 ± 0,04	0.322 ±0.011	7,069 ± 1,709	0,703 ± 0,076	0,021 ± 0,003	335	368
	Sp2	1,30 ± 0,07	0.366 ±0.016	7,576 ± 0,496	0,640 ± 0,008	0,011 ± 0,001	681	739
	Sp912	1,10 ± 0,04	0.355 ±0.042	6,423 ± 0,181	0,636 ± 0,072	0,011 ± 0,002	575	632
	Sp41	1,26 ± 0,10	0.347 ±0.014	6,446 ± 0,940	0,589 ± 0,053	0,009 ± 0,002	696	759
	Adana99	0,90 ± 0,01	0.359 ±0.021	6,630 ± 0,275	0,727 ± 0,025	0,011 ± 0,001	617	684
2000ppm NaCl 2500ppm NaCl	Sp732	0,68 ± -	55	7,116 ± -	0,840 ± -	0,561 ± -	13	14
	Sp563	0,42 ± -	37	6,954 ± -	0,783 ± -	0,394 ± -	18	20
	Sp804	0,44 ± -	30	7,596 ± -	0,782 ± -	0,438 ± -	17	19
	Sp757	0,35 ± 0,22	26	6,673 ± 0,636	0,869 ± 0,088	0,366 ± 0,065	25	28
	Sp492	0,29 ± 0,06	26	7,431 ± 0,138	1,042 ± 0,016	0,821 ± 0,089	9	10
	Sp2	0,24 ± 0,04	19	6,380 ± 0,593	1,115 ± 0,256	0,822 ± 0,154	8	9
	Sp912	0,20 ± 0,02	18	6,137 ± 0,454	1,288 ± 0,178	1,280 ± 0,075	5	6
	Sp41	0,21 ± 0,02	16	6,084 ± 0,851	1,326 ± 0,171	0,945 ± 0,225	6	8
	Adana99	0,27 ± 0,08	30	4,543 ± 0,374	0,967 ± 0,142	0,600 ± 0,107	8	9

Appendix 4 - Enzymatic Activity

Table. 7 - Protein concentration from the 5 selected genotypes studied on the third experiment.

Protein Concentration (mg g^{-1} FW)			
Condition	**Genotype**	**Protein Concentration ± STD**	**SE**
Control	Sp41	35.0 ± 4.5	13.0
	Sp67	33.2 ± 6.7	20.3
	Sp92	29.7 ± 2.1	7.2
	Sp96	25.5 ± 1.8	6.9
	Sp912	27.5 ± 4.4	16.1
Salt Stress	Sp41	32.8 ± 0.0	0.0
	Sp67	33.4 ± 5.2	15.6
	Sp92	31.1 ± 2.4	7.6
	Sp96	26.6 ± 5.6	20.9
	Sp912	35.9 ± 6.6	18.3

Table. 8 - SOD Activity from the 5 selected genotypes studied on the third experiment.

SOD Activity (U g^{-1} FW)			
Condition	**Genotype**	**SOD Activity ± STD**	**SE**
Control	Sp41	44.0 ± 1.4	3.2
	Sp67	46.2 ± 1.2	2.6
	Sp92	45.0 ± 5.0	11.0
	Sp96	40.8 ± 7.3	17.8
	Sp912	43.3 ± 3.3	7.7
Salt Stress	Sp41	49.7 ± 0.0	0.0
	Sp67	48.6 ± 1.1	2.3
	Sp92	49.3 ± 1.9	3.9
	Sp96	45.8 ± 2.4	5.2
	Sp912	48.6 ± 1.9	3.9

Table. 9 - GR Activity from the 5 selected genotypes studied on the third experiment.

Glu. Red. Activity (µmol [NADPH] g^{-1} FW min^{-1})					
Condition	**Genotype**	**Glu. Red. Activity ± STD**			**SE**
Control	Sp41	22.3	±	9.2	41.2
	Sp67	16.3	±	2.0	12.1
	Sp92	15.2	±	2.1	13.6
	Sp96	11.7	±	1.6	13.5
	Sp912	12.9	±	1.0	7.9
Salt Stress	Sp41	17.2	±	0.0	0.0
	Sp67	15.8	±	0.5	3.1
	Sp92	15.5	±	1.1	7.0
	Sp96	12.2	±	1.8	14.8
	Sp912	15.8	±	1.5	9.2

Table. 10 - CAT Activity from the 5 selected genotypes studied on the third experiment.

Catalase Activity (µmol H_2O_2 g^{-1} FW min^{-1})					
Condition	**Genotype**	**Catalase Activity ± STD**			**SE**
Control	Sp41	2538	±	749	30
	Sp67	2758	±	506	18
	Sp92	2474	±	492	20
	Sp96	1969	±	134	7
	Sp912	1813	±	142	8
Salt Stress	Sp41	2283	±	0	0
	Sp67	2424	±	603	25
	Sp92	2596	±	554	21
	Sp96	2783	±	734	26
	Sp912	2505	±	635	25

Table. 11 - APX Activity from the 5 selected genotypes studied on the third experiment.

Ascorbate P. Activity ($\mu mol\ H_2O_2\ g^{-1}\ FW\ min^{-1}$)					
Condition	**Genotype**	**Ascorbate P. Activity ± STD**			**SE**
Control	Sp41	37.0	±	4.3	11.5
	Sp67	37.6	±	5.4	14.4
	Sp92	33.4	±	2.4	7.0
	Sp96	31.3	±	1.8	5.9
	Sp912	32.0	±	1.6	4.9
Salt Stress	Sp41	41.9	±	0.0	0.0
	Sp67	44.9	±	6.3	14.1
	Sp92	41.7	±	4.5	10.8
	Sp96	39.0	±	3.3	8.4
	Sp912	39.5	±	2.6	6.6